

Standardization of results obtained with different thromboplastin preparations

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156 Figures, 82 Tables



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SUPPLEMENTUM XXXIX AD THROMBOSIS ET DIATHESIS HAEMORRHAGICA

32. Standardization of Results Obtained with Different Thromboplastin Preparations

E. A. LOELIGER, W. K. TACONIS, J. J. VELTKAMP, and H. C. HEMKER

It is beyond doubt that the ratio method conceived and worked out by Biggs and Denson (1) is invaluable for daily practice and even more so for clinical research. At the 1967 meeting of the Subcommittee of Thromboplastin Standards in Washington, however, it was clear that four relevant problems were still unsolved (8, 10). These problems concern: 1. The theoretical basis of the ratio method; 2. the statistical validity of the ratio method; 3. the definition of an absolute standard; 4. the proposal of a standard reference preparation. Since 1967, we have increased our experience and feel able to clarify some of these problems.

Theoretical basis

Biggs and Denson have put forward strong arguments in favor of the hypothesis that the correlation between the ratios found with two different thromboplastin preparations in plasmas from patients treated with coumarin congeners is rectilinear and that the line goes through the point defined by ratio 1 of the two axes of the diagram. Rectilinearity is assumed to be the result of rectilinearity of the underlying correlations of coagulation time ratios with the inverse of the concentration of coagulation factors present in the coumarin plasma, a correlation known from normal plasma containing different amounts of coagulation factors (2, 4).

The hypothetical intersection at the point ($x = 1$, $y = 1$) defined in the ratio plot is based on the assumption that the correlation curves obtained from the two thromboplastin preparations display the same behavior as do, by definition, the lines obtained from normal plasma.

Concerning the first assumption, we now have enough evidence to be certain that in plasma from coumarin-treated patients (under the condition of stable hypo-coagulability) the correlation between the coagulation time ratios and the inverse of the concentration of the coagulation factors involved is indeed rectilinear, provided the thromboplastin preparation used is free of important factor VII-like

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activity. The most convincing curves are obtained with Thrombotest (Fig. 1) and with Simplastin (Fig. 2).

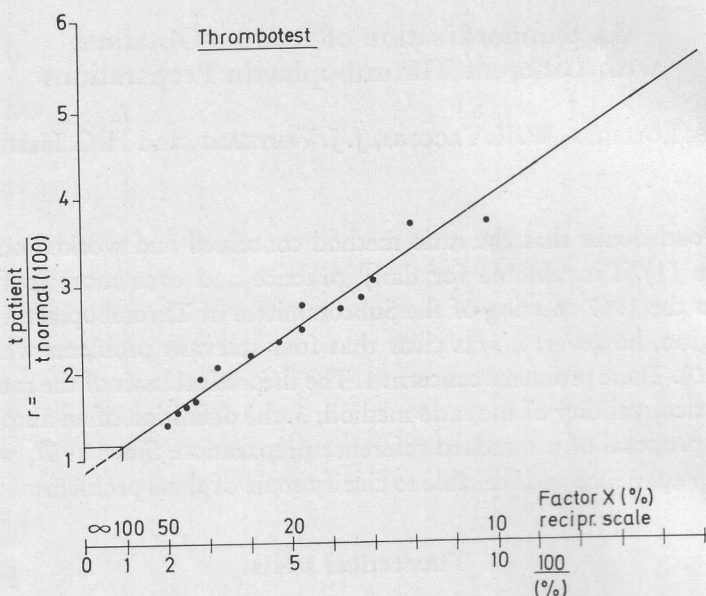


Fig. 1. Rectilinear correlation between coagulation time ratios found with Thrombotest and the inverse of the factor X concentration in patients' plasma. The scale of the y-axis on which the ratios are plotted is normal; the x-axis, on which the factor X concentrations are plotted, has a reciprocal scale. It must be stressed that each point represents a result obtained from pooled patient plasma, each pool consisting of equal amounts of ten plasmas from patients stabilized at a certain level of anticoagulation; the levels of the 15 pools differed, in terms of factor X, between 10% and 50% of normal. Factor X has been assessed by the enzyme kinetic approach described by Hemker (5). Similar if not identical results were obtained by correlating ratios with factor II or factor IX (both assessed by conventional one-stage assay procedures).

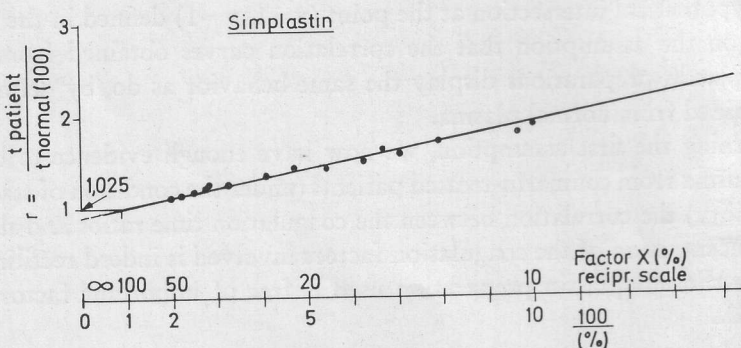


Fig. 2. Results obtained with Simplastin in samples of the same 15 plasmas.

Re-examination of the curve obtained with Thrombotest (Fig. 3) shows not only rectilinearity but also that the correlation line is much steeper than the corresponding line obtained from normal plasma. (Coagulation time ratios obtained when testing normal plasma in different dilutions with buffer, e.g., a $1/10$ dilution corresponds to 10 % factor X.) The line does not pass through point ($x=1$, $y=1$) defined in the ratio plot as does, by definition, the line obtained from normal plasma.

In the patient, point 1 of the x-axis (= 100 % factor X) corresponds to a virtual ratio (or y-value) of 1.17. This point is found by rectilinear extrapolation of the correlation line found for therapeutic values. (Virtual ratio means the ratio expected in a patient which under the condition of stable coumarin treatment displays 100 % factor X activity which, in practice, is not expected to occur.)

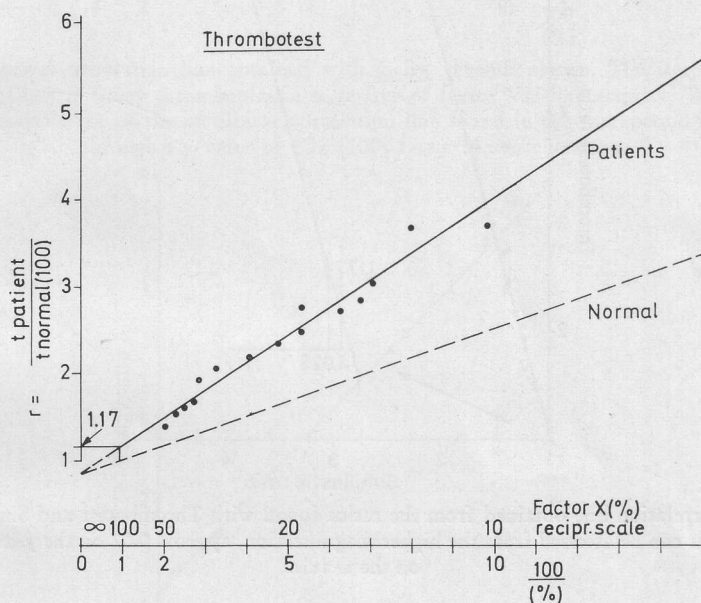


Fig. 3. The correlation lines found with Thrombotest for coumarin plasma and normal plasma. Both intersect the y-axis at the same point. At 100 % factor X activity, the (virtual) ratio for patients is 1.17.

This upward deviation of the line found in patients as compared to the correlation line obtained from normal plasma is not at all astonishing, if we consider that coumarin congeners not only induce a depression of the four factors of the prothrombin complex, but also provoke the appearance in the circulation of a protein, called PIVKA, that acts as a competitive inhibitor of the thromboplastin time reaction and whose concentration is independent of the level of hypocoagulability (5).

Differences in PIVKA sensitivity of the different thromboplastin preparations may cause more or less obvious deviations from the ratio correlation line proposed by Biggs and Denson. The deviations found, however, are minimal: for instance, if the ratios found with Thrombotest, which is highly sensitive to both a coagulation factor deficiency and PIVKA, are correlated with the ratios obtained for Simplastin, which displays a relatively low sensitivity to the coagulation factor deficiency, then, in spite of the very low PIVKA sensitivity of Simplastin, the deviation is minimal (Fig. 4).

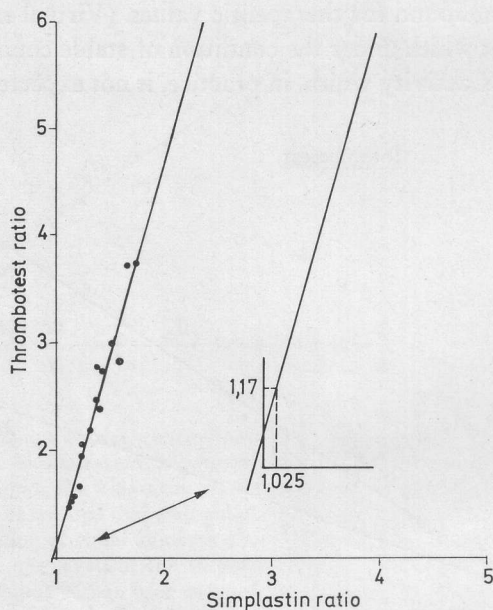


Fig. 4. The correlation line obtained from the ratios found with Thrombotest and Simplastin. The deviation is, as can be assessed from the higher magnification, approx. 0.07 on the y-axis and 0.015 on the x-axis.

Another cause of deviation from the correlation line proposed by Biggs and Denson is the non-rectilinearity of the correlation line obtained with thromboplastins possessing important factor VII-like activity. With such thromboplastins the correlation lines may be distinctly curved and show flattening toward the right, the part of the curve falling in the therapeutic range of coumarin-induced hypo-coagulability being rectilinear, however. The line found for Geigy thromboplastin is an example of this kind of behavior, suggesting a curved shape of the line between [100] and [40] and showing rectilinearity in the therapeutic range (Fig. 5).*

* The curved shape is well known from the standard reference curve.

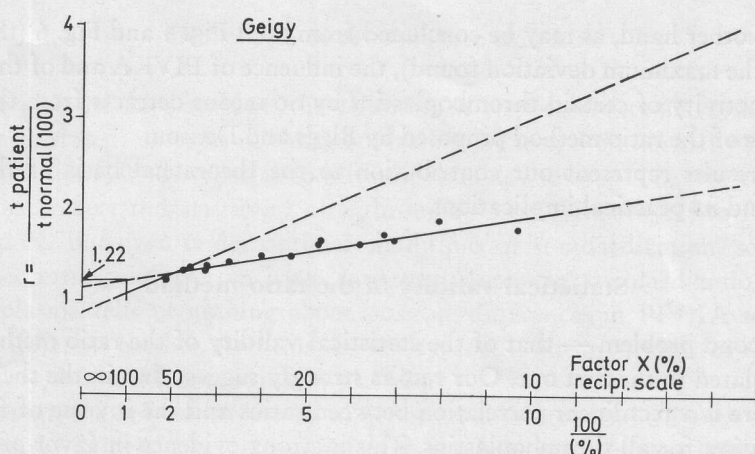


Fig. 5. The curved correlation line obtained with Geigy thromboplastin. The steep dotted lines would be obtained if Geigy thromboplastin were free of factor VII-like activity. The flat dotted line is an extrapolation of the rectilinear correlation line found in the therapeutic range: it goes through a y-value of 1.22 [100] factor X concentration.

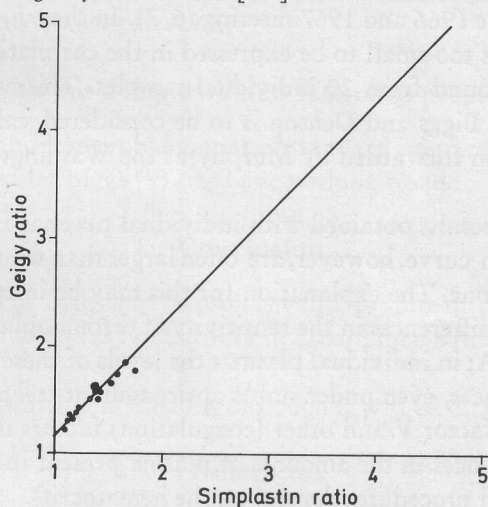


Fig. 6. Correlation line obtained from ratios found with Geigy thromboplastin and Simplastin. The line is rectilinear, but obviously does not pass through point (1, 1); at $x = 1$, y is about 1.20.

Although, in the therapeutic range, the steepness of the line found for Geigy thromboplastin is very similar to that found for Simplastin, the ratios are clearly different; the virtual y-values at [100] factor X (obtained by extrapolating the lines found for the therapeutic range) for Geigy thromboplastin and Simplastin are 1.22 and 1.025, respectively. This difference causes deviations from the behavior postulated by Biggs and Denson, and these deviations are clearly larger than those caused by PIVKA (Fig. 6).

On the other hand, as may be concluded from both Fig. 4 and Fig. 6 (the latter showing the maximum deviation found), the influence of PIVKA and of the factor VII-like activity of certain thromboplastins by no means detracts from the practical value of the ratio method proposed by Biggs and Denson.

These results represent our contribution to the theoretical basis of the ratio method and its practical implications.

Statistical validity of the ratio method

The second problem — that of the statistical validity of the ratio method — is closely related to the first one. Our results strongly suggest that in the therapeutic range there is a rectilinear correlation between ratios and the inverse of factor X concentration for all thromboplastins. This is strong evidence in favor of a rectilinear correlation between the various ratios, too. Deviations from the hypothetical intersection at point ($x=1$, $y=1$) through the influence of PIVKA of factor VII-like activity of thromboplastin may explain the criticism put forward by Littell and Ratnoff at the 1966 and 1967 meeting (6, 7). In the majority of cases, however, this deviation is too small to be expressed in the correlation line constructed through the points found from 20 individual samples. This means that the ratio method proposed by Biggs and Denson is to be considered valid for all practical purposes, a conclusion forwarded by Murphy at the Washington meeting in 1967 (3).

Deviations of the results obtained with individual plasmas from the best-fitting rectilinear correlation curve, however, are often larger than would be expected from the technical error alone. The explanation for this may be in one of the following three possibilities: a. differences in the sensitivity of thromboplastins for factors VII and X and for PIVKA; in individual plasmas the levels of these factors show rather large mutual differences, even under stable anticoagulant treatment; b. differences in the sensitivity to factor V and other (coagulation) factors in prothrombin time estimations; c. differences in the amounts of plasma present in the test system (the result of whole-blood procedures depend on the hematocrit).

Deviations are especially large when results obtained with human brain and animal material are compared. Although the underlying cause is not yet fully understood, it may be an exceptional factor VII sensitivity of human brain thromboplastin.

To eliminate the influence of these rather large deviations as much as possible, the number of samples tested should be higher than 20, which means that the help of a specialized reference laboratory would often be necessary.

Definition of an absolute standard

Concerning the third problem, the definition of an absolute standard, the following may be stated: at present, thromboplastins are tested on normal plasma and plasma dilutions. Under special conditions, standard reference curves can be obtained that reflect the sensitivity of a thromboplastin with respect to factors II, VII, and X. But even under optimal conditions of standardization, sensitivity, and hence ratios, differ from batch to batch. Moreover, standardization against normal plasma tells us nothing about possible differences in PIVKA sensitivity between different batches. And this sensitivity, too, is all-important for the results (c.q. ratios) obtained from coumarin plasmas.

At present, the only sensible way to define an absolute standard with respect to the coumarin-induced coagulation defect as a whole is to prepare large pools of coumarin plasma containing known amounts of coagulation factors. We are fully aware of the fact that such standardization needs exceptional facilities. We think, however, that our Thrombosis Service would be able to satisfy many of the prerequisites for absolute standardization.

Proposal for a standardized reference preparation

As to the fourth and last problem, that of standard preparations, which has been extensively discussed by Biggs (9), we have nothing to add.

Conclusion

The ratio method of Biggs and Denson is valid, in spite of the fact that the theoretical basis of this method is not entirely in accordance with the original assumptions.

Absolute standardization is feasible, although it will be difficult to realize.

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